

1 **MicroRNA-27a-5p inhibits proliferation, migration and invasion and promotes**
2 **apoptosis of Wilms tumor cell by targeting PBOV1**

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13 **Running head:** MicroRNA-27a-5p inhibits proliferation of Wilms tumor cell

14 **Abbreviations**

15 miRNA: microRNA; 3'-UTR: 3'-Untranslated region; ATCC: American Type Culture
16 Collection; PI: Propidium iodide; WT: Wilms tumor.

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24 **Introduction**

25 Wilms tumor is the commonest renal carcinoma mostly happened in children under
26 age 5(Rivera and Haber, 2005). The survival of Wilms tumor patients has been
27 significantly improved from less than 30% to more than 90% in the past decade due to
28 modern therapeutic strategies and technology(Szycho et al., 2014; Lopes and Lorenzo,
29 2017). However, the current therapies such as radiotherapy and chemotherapy have
30 serious side effects with poor efficacy in patients with tumor metastasis(Pritchard-Jones,
31 2002; Ehrlich et al., 2009; Akakin et al., 2016). Meanwhile, large-scale next-generation
32 sequencing has identified multiple mutations of candidate driver in Wilms tumor(Gadd et
33 al., 2017). Thus, it is important to further understanding the molecular mechanism of
34 Wilms tumor oncogenesis and metastasis and develop new treatment strategies.

35 MicroRNAs (MiRNAs) are small non-coding RNAs that play crucial regulatory roles
36 in various biological processes including tumorigenesis(Peng and Croce, 2016; O'Brien et
37 al., 2018). MiRNAs post-transcriptionally regulate their target gene expression via
38 binding to the 3'-UTR of target mRNAs(O'Brien et al., 2018). The expression and
39 function of miRNAs in Wilms tumor have also been investigated(Yu et al., 2016).
40 MiRNA microarray profiling results from 36 Wilms tumor of different subtypes and
41 normal kidney tissues demonstrated that various miRNAs were dysregulated in blastema
42 Wilms tumor and regressive subtype of Wilms tumor(Ludwig et al., 2016). Those
43 miRNAs function as oncogenes, tumor suppressors, or mediate the chemo-sensitivity in
44 Wilms tumor(Ludwig et al., 2016). Watson JA et al. also reported that miRNAs could
45 predict the chemo-responsiveness in Wilms tumor blastema, with 29 miRNAs identified
46 to be markedly differentially expressed in post-treatment high-risk and intermediate-risk

47 patients(Watson et al., 2013). While miR-483-3p functions as an oncogene and promotes
48 the development and chemo-resistance of Wilms tumor, miR-27 was reported to be
49 downregulated in Wilms tumor(Watson et al., 2013; Wegert et al., 2015). However, the
50 detailed functional role and molecular mechanisms of miR-27 in Wilms tumor are not
51 fully understood.

52 In this study, we evaluated the expression profile and function of miR-27-5p in
53 Wilms tumor and cell lines. The results demonstrated that miR-27a-5p was low-
54 expressed in human Wilms tumor tissues and cells. Overexpression of miR-27a-5p
55 inhibited cell proliferation, cell migration and invasion and promoted cell apoptosis.
56 Moreover, our data revealed that miR-27a-5p suppressed tumorigenesis via negatively
57 regulating PBOV1. In summary, our findings suggest that miR-27a-5p might serve as a
58 novel therapeutic target in Wilms tumor.

59 **Materials and Methods**

60 **Patient specimens**

61 Twenty pairs of Wilms tumor and adjacent normal kidney tissues were obtained from
62 patients who underwent surgery at the Second Affiliated Hospital of Xi'an Jiaotong
63 University (Xibe Hospital) between March 2019 and September 2019. All Wilms tumor
64 tissues were histopathologically confirmed and classified based on the American National
65 Wilms Tumor Study 5 typing and TNM staging system. Tissues were snap-frozen and
66 stored in liquid nitrogen until further analysis. The study was reviewed and approved by
67 the Ethics Committee of Xibe Hospital and all patients provided the written informed
68 consent.

69 **Cell culture**

70 Wilms tumor-derived renal cancer cell line WiT49, STA-WT3ab, RM1, PSU-SK-1
71 and control HEK 293T cells were purchased from American Type Culture Collection
72 (ATCC, USA). Cells were maintained in DMEM medium supplemented with 10% fetal
73 bovine serum (Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) at 37 °C in a
74 5% CO₂ incubator.

75 **Transfection**

76 Transfection was conducted using Lipofectamine 2000 (Invitrogen, USA). MiR-27a-
77 5p mimic or negative control (NC), miR-27a-5p inhibitor and NC were obtained from
78 GenePharma (Shanghai, China). PBOV1 siRNA and scramble control were purchased
79 from Rubibio Company (Guangzhou, China). pcDNA 3.1-PBOV1 was obtained from
80 Genecopoeia (Maryland, USA).

81 **Lentivirus infection and generation of stable cell line**

82 To generate stable cell line overexpressing miR-27a-5p or miR-NC, WiT49 cells
83 were infected with lentivirus-miR-27a-5p mimic or lentivirus-miR-NC lentivirus particles.
84 Lentivirus-miR-NC vector or lentivirus-miR-27a-5p mimic vector, together with the
85 helper plasmids pHelper 1.0 (Gag and Pol) and pHelper 2.0 (VSVG) were transiently
86 cotransfected into HEK 293T cells using Lipofectamine 2000 (Invitrogen, USA) to
87 package lentivirus particles, respectively. When WiT49 cells were grown in the
88 logarithmic growth phase, cells were infected with lentivirus particles with a multiplicity
89 of infection of 70. Then, limited dilution method was used and cells were cultured with
90 puromycin (2 µg/mL) and screened for 14 days to select the stable cell lines
91 overexpressing miR-27a-5p or miR-NC.

92 **RT-qPCR**

93 To analyze the relative expression level of miR-27a-5p and PBOV1, RNA was
94 purified from Wilms tumor tissues or cultured cells using a miRNeasy Kit (Qiagen,
95 German) and then reverse transcribed into cDNA using MiR-X miRNA Synthesis kit
96 (Clontech, USA) or SuperTranscriptase III (Invitrogen, USA) following the
97 manufacturers' instructions. Quantitative PCR was conducted using the SYBR Green mix
98 (Takara, Japan) on a Bio-Rad Real-time CFX96 system. U6 snRNA and GAPDH were
99 used as the internal control. The gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

100 The following primers were used: hsa-miR-27a-5p: 5'-
101 AGGGCTTAGCTGCTTGTGAGC-3'; hsa-PBOV1-Forward: 5'-
102 AGTTCGAGACCAGCCTGACCAG-3', hsa-PBOV1-Reverse: 5'-
103 TTCAAGCAATTCTCCGCCTCAGC-3'; hsa-SPARC-Forward: 5'-
104 CAAGAAGCCCTGCCTGATGAGAC-3', hsa-SPARC-Reverse: 5'-
105 TTCCGCCACCACCTCCTCTTC-3'; hsa-GSDMA-Forward: 5'-
106 ACGCTGCTGGATGTGCTTGAG-3', hsa-GSDMA-Reverse: 5'-
107 AGAGCCCTGCCGTTCCCTTC-3'; hsa-ASB15-Forward: 5'-
108 GCCTGGACATTAGGTTTGACC-3', hsa-ASB15-Reverse: 5'-
109 GTCAGGGGAGCCAGATAAGC-3'; hsa-UBXN4-Forward: 5'-
110 CCTTCTGATGCTCCTCTAGAAG-3', hsa-UBXN4-Reverse: 5'-
111 GGAAACATGGTTGCTAACGAAA-3'.

112 **Western blot**

113 Protein was prepared from tumor tissues or cultured cells using RIPA buffer
114 (Beyotime, China) and quantified with a BCA protein assay kit (Pierce, USA). Western

115 blot was performed using a standard protocol with primary antibodies against PBOV1
116 (Abcam, ab216045), β -actin (Abcam, ab82227). β -actin was used as a loading control.

117 **Luciferase reporter assay**

118 Luciferase reporter vectors containing WT or mutated 3'-UTR of PBOV1 were
119 constructed based on the backbone vector pGL3-Luc. WiT49 cells were co-transfected
120 with reporter vectors, and miR-NC or miR-27a-5p mimic. After 48 hours, luciferase
121 activity was measured with a Dual-luciferase reporter assay kit (Promega, USA).

122 **CCK-8 assay**

123 Transfected WiT49 or STA-WT3ab cells were cultured in 96-well plates and 10 μ l
124 Cell Counting Kit-8 (CCK-8, Dojindo, Japan) reagent was added and cultured for 2 more
125 hours. The absorbance (450 nm) was recorded 2 hours later.

126 **Cell apoptosis assay**

127 Transfected WiT49 or STA-WT3ab cells were collected and stained with a BD
128 apoptosis analysis kit (BD Bioscience, USA). After staining, cells were analyzed using
129 the Cytoflex flow cytometry machine (Beckman Coulter), and the Annexin V+
130 Propidium iodide – cells were defined as apoptotic cells. The results were analyzed by
131 Flowjo (Treestar, USA).

132 **Transwell assay**

133 Transfected WiT49 or STA-WT3ab cells were resuspended in serum-free medium
134 and seeded to the top chamber (Corning, USA) with or without pre-coating of Matrigel
135 (BD Bioscience, USA). Complete medium with 10% FBS was added to the bottom
136 chamber. After culture for 24 hours, the invaded or migrated cells were stained with 0.1%
137 crystal violet (Solarbio, China) and counted.

138 **Xenograft tumor model**

139 Ten male BALB/c nude mice (5-6 weeks old) were purchased from SLAC animal
140 center (Shanghai, China) and randomly divided into 2 groups. WiT49 cells with stable
141 overexpressing miR-27a-5p mimic or miR-NC were inoculated subcutaneously into nude
142 mice, respectively. Tumor growth was recorded at indicated time points and calculated:
143 $\text{Volume} = \text{length} \times \text{width}^2/2$. Mice were sacrificed and analyzed on day 22. The
144 experiments were approved by the Animal Care Committee of Xibei Hospital.

145 **Statistical analysis**

146 Results were shown as mean \pm standard deviation (SD) from three independent
147 experiments and analyzed by using GraphPad Prism V7.0 (Prism, USA). Student *t*-test
148 and one-way analysis of variance (ANOVA) were conducted where necessary. A *p* <
149 0.05 was defined as statistically significant.

150 **Results**

151 **MiR-27a-5p is downregulated in human Wilms tumor tissues and cells**

152 To determine the expression of miR-27a-5p in Wilms tumor, we performed qPCR to
153 examine the miR-27a-5p expression in twenty pairs of Wilms tumor tissues and adjacent
154 control tissues (**Fig 1A**). MiR-27a-5p expression was significantly decreased in human
155 Wilms tumor tissues (**Fig 1A**). Consistently, MiR-27a-5p expression was measured in
156 different Wilms tumor cell lines and the results showed that Wilms tumor cell lines
157 (WiT49 and STA-WT3ab) had markedly lower levels of miR-27a-5p than that in control
158 cell line HEK 293T (**Fig 1B**).

159 **MiR-27a-5p inhibits proliferation, migration and invasion and promotes apoptosis** 160 **in Wilms tumor cells**

161 Then we performed functional assays to assess the role of miR-27a-5p in Wilms
162 tumor cells. WiT49 and STA-WT3ab cells, which had relatively lower expression of
163 miR-27-5p, were transfected with miR-27a-5p mimic to overexpress miR-27-5p (**Fig 2A**).
164 Overexpression of miR-27a-5p remarkably repressed cell proliferation (**Fig 2B**) and
165 promoted cell apoptosis (**Fig 2C**) in WiT49 and STA-WT3ab cells. Furthermore,
166 compared with the negative control, miR-27a-5p mimic transfection significantly
167 suppressed cell migration and invasion (**Fig 2D** and **2E**). These findings suggest that
168 miR-27a-5p negatively regulates Wilms tumor development.

169 **MiR-27a-5p inhibits oncogenesis and metastasis of Wilms tumor in vivo**

170 To verify the tumor suppressor role of miR-27-5p, in vivo xenograft model was
171 established in nude mice by subcutaneous injection of WiT49 cells transfected with miR-
172 27a-5p mimic or control miRNA. The results demonstrated the tumor inhibitory function
173 of miR-27a-5p in vivo. MiR-27a-5p overexpression significantly inhibited Wilms tumor
174 development (**Fig 3A**). Tumors developed from the miR-27a-5p mimic group showed a
175 much smaller size, with a lower tumor weight in comparison with those developed from
176 control cells (**Fig 3B** and **3C**). The upregulated miR-27a-5p expression was confirmed in
177 tumors from the miR-27a-5p mimic group (**Fig 3D**).

178 **PBOV1 is a direct target of miR-27a-5p in Wilms tumor cells**

179 To explore the potential targets regulated by miR-27a-5p, we performed
180 bioinformatics analysis using different online databases (TargetScan, miRDB, and
181 miRWalk). As shown in **Fig 4A**, five genes including PBOV1, SPARC, ASB15, UBXN4,
182 and GSDMA were predicted to the potential targets of miR-27a-5p. WiT49 cells were
183 transfected with miR-NC or miR-27a-5p mimic and the relative expression of these

184 potential targets was analyzed. PBOV1 was markedly downregulated by miR-27a-5p
185 mimic (**Fig 4B**). In addition, miR-27a-5p had the putative binding sequences against 3'-
186 UTR of the PBOV1 gene (**Fig 4C**). Luciferase reporter assay further validated the
187 interaction between miR-27a-5p and WT 3'-UTR of PBOV1, as miR-27a-5p markedly
188 inhibited the luciferase activity of reporter vector containing WT 3'-UTR of PBOV1 (**Fig**
189 **4D**). Moreover, we demonstrated that miR-27a-5p mimics significantly inhibited the
190 mRNA and protein levels of PBOV1 in WiT49 or STA-WT3ab cells (**Fig 4E** and **4G**). In
191 the contrast, inhibition of miR-27a-5p enhanced the expression of PBOV1 in WiT49 or
192 STA-WT3ab cells (**Fig 4F** and **4H**).

193 **Knockdown of PBOV1 suppresses cell migration and invasion and promotes cell** 194 **apoptosis of Wilms tumor cells**

195 We found that Wilms tumor tissues had a much higher expression level of PBOV1
196 compared with adjacent normal tissues (**Fig 5A**). Similarly, we detected higher
197 expression of PBOV1 in Wilms tumor cells (WiT49 and STA-WT3ab) compared with
198 that in control HEK 293T cells (**Fig 5B**). To study the function of PBOV1, siRNA-
199 targeting PBOV1 was used to suppress the expression of PBOV1 in WiT49 or STA-
200 WT3ab cells. The knockdown efficiency was evaluated by western blot (**Fig 5C**).
201 Functionally, we demonstrated that knockdown of PBOV1 suppressed cell proliferation
202 and enhanced apoptosis in WiT49 or STA-WT3ab cells (**Fig 5D** and **5E**). While transwell
203 assay revealed that inhibition of PBOV1 decreased the capability of cell migration and
204 invasion in WiT49 or STA-WT3ab cells (**Fig 5F** and **5G**). Thus, the results suggested
205 that PBOV1 acted as an oncogene in Wilms tumor.

206 **Overexpression of PBOV1 antagonizes the tumor suppressor function of miR-27a-**
207 **5p in Wilms tumor cells**

208 To validate the functional relationship between miR-27a-5p and PBOV1, rescue
209 experiments were performed. WiT49 or STA-WT3ab cells were transfected with miR-
210 NC, miR-27a-5p mimic, or miR-27a-5p mimic+pcDNA-PBOV1. Whereas miR-27a-5p
211 overexpression significantly decreased the expression of PBOV1, overexpression of
212 PBOV1 together with miR-27a-5p rescued PBOV1 expression in Wilms tumor cells (**Fig**
213 **6A**). Functionally, miR-27a-5p mimic inhibited cell proliferation and PBOV1
214 overexpression abrogated the inhibitory effect of miR-27a-5p (**Fig 6B**). Conversely, miR-
215 27a-5p enhanced cell apoptosis of WiT49 or STA-WT3ab cells. Overexpression of
216 PBOV1 together with miR-27a-5p mimic showed reduced cell apoptosis and was
217 comparable to that in cells transfected with miR-NC control (**Fig 6C**). In addition,
218 overexpression of PBOV1 antagonized the inhibitory effect on cell migration and
219 invasion of miR-27a-5p in WiT49 or STA-WT3ab cells (**Fig 6D** and **6E**). Taken together,
220 the data suggested miR-27a-5p exerted its tumor-suppressive role in Wilms tumor cells
221 via downregulating PBOV1.

222 **Discussion**

223 Studies have shown that miRNAs play essential roles in tumorigenesis and
224 metastasis(Si et al., 2019). MiRNAs also exerts the regulatory function in Wilms tumor
225 and could be used as diagnostic markers and predictors for chemo-
226 responsiveness(Schmitt et al., 2012; Watson et al., 2013). Here we reported that miR-
227 27a-5p was low-expressed in Wilms tumor and miR-27a-5p overexpression suppressed
228 Wilms tumor cell growth and metastasis. PBOV1 was demonstrated to be the direct target

229 of miR-27a-5p and overexpression of PBOV1 abrogated the tumor-suppressive function
230 of miR-27a-5p. Thus, our findings suggest a potential therapeutic target of miR-27a-5p in
231 Wilms tumor patients.

232 MiR-27a-5p has been reported to suppress the tumorigenesis of multiple cancers
233 including prostate cancer, small cell lung cancer, and cervical adenocarcinoma(Mizuno et
234 al., 2017; Barros-Silva et al., 2018; Fang et al., 2018). Networks analysis showed that
235 miR-27a-5p was dysregulated in Wilms tumor(He et al., 2016). In another study, Jenny A.
236 Watson et al. showed that down-regulation of miR-27a was found in the high-risk Wilms
237 tumors, which might be a predictor of chemo-responsiveness(Watson et al., 2013). We
238 confirmed that miR-27a-5p was low-expressed in Wilms tumor. Consistent with the
239 published data, the tumor-suppressive function of miR-27a-5p was validated both in vitro
240 and in vivo. As overexpression of miR-27a-5p inhibited the growth and metastasis of
241 Wilms tumor cells and promoted cell apoptosis.

242 Bioinformatics analysis predicted multiple potential targets of miR-27a-5p while we
243 verified that miR-27a-5p mimics specifically inhibited the expression of PBOV1. PBOV1
244 was first identified as a human tumor-specific gene and associated with the clinical
245 outcome of cancer patients(Krukovskaia et al., 2010; Samusik et al., 2013). The high
246 expression level of PBOV1 promoted G1/S transition and enhanced cell proliferation in
247 prostate cancer(Pan et al., 2016). The function of PBOV1 was also elucidated in
248 hepatocellular carcinoma and overexpression of PBOV1 was correlated with poor
249 prognosis of HCC patients, indicating PBOV1 as a prognostic biomarker of HCC(Xue et
250 al., 2018). However, there are few reports regarding the regulation of PBOV1 in tumors.
251 Zhang SY et al demonstrated PBOV1was regulated by miR-203 in fracture

252 healing(Zhang et al., 2018). In rheumatoid arthritis, monocyte differentiation was
253 controlled by lncRNA NTT/PBOV1 axis(Yang et al., 2018). In this study, we reported
254 for the first time that PBOV1 was directly regulated by miR-27a-5p and PBOV1
255 overexpression antagonized the function of miR-27a-5p.

256 Though we demonstrated that miR-27a-5p/PBOV1 axis regulated Wilms tumor
257 development and progression with both in vitro and in vivo evidence, there are several
258 limitations in this study. First, it is of great importance to further study whether high
259 expression of miR-27a-5p mediated the chemo-resistance in Wilms tumor or not. Second,
260 whether there are other potential miRNAs involved in PBOV1 regulation remain
261 unknown. Additionally, the signaling pathways involved in miR-27a-5p/PBOV1 axis in
262 Wilms tumor need further investigation.

263 **Conclusion**

264 MiR-27a-5p acts as a tumor suppressor via negatively regulating PBOV1 in Wilms
265 cancer. Our data suggest that miR-27a-5p/PBOV1 might be utilized as a novel
266 therapeutic target in Wilms tumor.

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268 **Authors' contributions**

269 Zheng-Tuan Guo and Qiang Yu conceived and designed these experiments. Chunlin
270 Miao, Wenan Ge and Peng Li performed these experiments. Qiang Yu and Wenan Ge
271 analyzed and interpreted the data. Chunlin Miao and Peng Li wrote the manuscript.
272 Zheng-Tuan Guo and Qiang Yu revised the manuscript. All authors read and approved
273 the final manuscript.

274

275 **Ethics approval and consent to participate**

276 The ethics committee of the Second Affiliated Hospital of Xi'an Jiaotong University
277 approved the study. The investigation conforms to the principles outlined in the
278 Declaration of Helsinki and written informed consent was obtained from all participants.

279 **Availability of data and material**

280 The datasets used and/or analyzed during the current study available from the
281 corresponding author on reasonable request.

282 **Disclosure statement**

283 The authors declare that they have no competing interests.

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287 **Reference**

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389 **Figure legends**

390 **Figure 1. MiR-27a-5p expression in Wilms tumor tissues and cell lines.**

391 (A) Relative expression of miR-27a-5p was determined in twenty pairs of Wilms tumor
392 tissues and adjacent normal control tissues by qPCR. (B) Relative expression of miR-
393 27a-5p was determined in Wilms tumor cell lines (WiT49, STA-WT3ab, RM1, and PSU-
394 SK-1) and control cell line HEK 293T by qPCR. ** $p < 0.01$.

395 **Figure 2. MiR-27a-5p inhibits proliferation, migration and invasion and promotes**

396 **apoptosis in Wilms tumor cells.** WiT49 or STA-WT3ab cells were transfected with
397 miRNA negative control (miR-NC) or miR-27a-5p mimic. (A) The relative expression of
398 miR-27a-5p in WiT49 or STA-WT3ab cells was analyzed by qPCR. (B) Cell
399 proliferation was analyzed at indicated time points by CCK8 assay. (C) Cell apoptosis
400 was analyzed by Annexin V/PI staining and flow cytometry. (D, E) Cell migration and
401 invasion were assessed by transwell assay. * $p < 0.05$, ** $p < 0.01$ vs. miR-NC.

402 **Figure 3. MiR-27a-5p inhibits Wilms xenograft tumor growth in vivo.** WiT49 cells

403 were transfected with negative control miRNA (miR-NC) or miR-27a-5p mimic and then
404 inoculated into BALB/c nude mice to develop the xenograft Wilms tumor. (A) Tumor
405 growth was monitored and measured at indicated time points. (B) Tumors were extracted
406 and recorded on Day 22. (C) Tumor weights of xenograft were analyzed. (D) The relative
407 expression of miR-27a-5p in xenograft tumors was analyzed by qPCR. * $p < 0.05$, ** $p <$
408 0.01 vs. miR-NC.

409 **Figure 4. PBOV1 is a direct target of miR-27a-5p in Wilms tumor cells.** (A)

410 Bioinformatics analysis was performed to predict the potential targets of miR-27a-5p
411 using online databases TargetScan, miRDB and miRWalk. (B) WiT49 cells were

412 transfected with miR-NC or miR-27a-5p mimic. The relative expression of PBOV1,
413 SPARC, ASB15, UBXN4 and GSDMA was analyzed by qPCR 48 hours later. (C) The
414 predicted binding sequences between miR-27a-5p and WT or mutated 3'-UTR of PBOV1.
415 (D) WiT49 cells were transfected with miR-NC or miR-27a-5p, together with luciferase
416 reporter vectors containing WT or mutated 3'-UTR of PBOV1. The relative luciferase
417 activity was analyzed 48 hours later. (E) WiT49 or STA-WT3ab cells were transfected
418 with miR-NC or miR-27a-5p mimic. The relative expression of PBOV1 mRNA was
419 analyzed by qPCR 48 hours later. (F) WiT49 or STA-WT3ab cells were transfected with
420 miR-NC or miR-27a-5p inhibitor. The relative expression of PBOV1 mRNA was
421 analyzed by qPCR 48 hours later. (G) WiT49 or STA-WT3ab cells were transfected with
422 miR-NC or miR-27a-5p mimic. The relative expression of PBOV1 protein was analyzed
423 by western blot 48 hours later. (H) WiT49 or STA-WT3ab cells were transfected with
424 miR-NC or miR-27a-5p inhibitor. The relative expression of PBOV1 protein was
425 analyzed by western blot 48 hours later. * $p < 0.05$, ** $p < 0.01$ vs. miR-NC.

426 **Figure 5. Knockdown of PBOV1 suppresses cell migration and invasion and**
427 **promotes cell apoptosis of Wilms tumor cells.** (A) The relative expression of PBOV1
428 mRNA in twenty pairs of Wilms tumor tissues and adjacent normal tissues was analyzed
429 by qPCR. (B) The relative expression of PBOV1 mRNA in Wilms tumor cell lines
430 (WiT49, STA-WT3ab, RM1, and PSU-SK-1) and control cell line HEK 293T was
431 analyzed by qPCR. (C-G) WiT49 or STA-WT3ab cells were transfected with negative
432 control (si-NC) or si-PBOV1. (C) The protein level of PBOV1 was analyzed by western
433 blot 48 hours post-transfection. (D) Cell proliferation was analyzed by CCK8 assay. (E)
434 Cell apoptosis was analyzed by Annexin V/PI staining and flow cytometry. (F, G) Cell

435 migration and invasion were assessed by transwell assay. * $p < 0.05$, ** $p < 0.01$ vs. si-
436 NC.

437 **Figure 6. Overexpression of PBOV1 antagonizes the tumor suppressor effect of**
438 **miR-27a-5p in Wilms tumor cells.** WiT49 or STA-WT3ab cells were transfected with
439 negative control (miR-NC), miR-27a-5p mimic, or miR-27a-5p mimic+pcDNA-PBOV1.
440 (A) The relative mRNA level of PBOV1 in WiT49 or STA-WT3ab cells was analyzed by
441 qPCR 48 hours post-transfection. (B) Cell proliferation was analyzed by CCK8 assay at
442 indicated time points. (C) Cell apoptosis was analyzed by Annexin V/PI staining and
443 flow cytometry. (D, E) Cell migration and invasion were assessed by transwell assay. * p
444 < 0.05 , ** $p < 0.01$.

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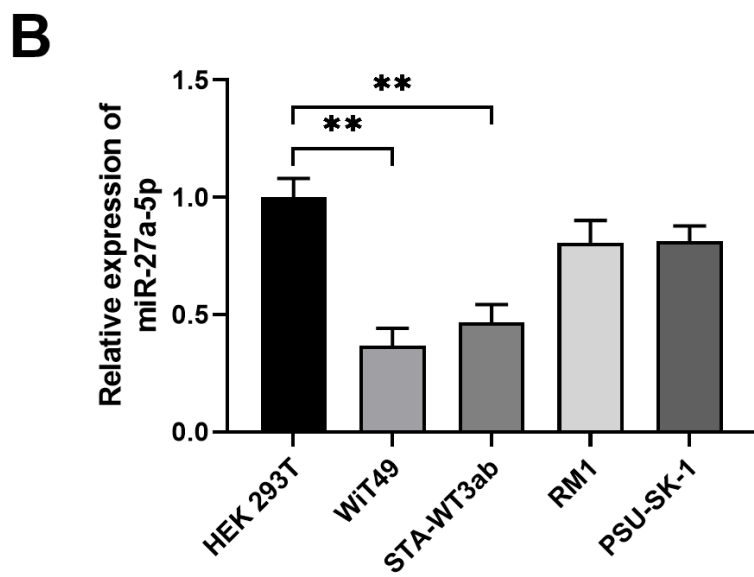
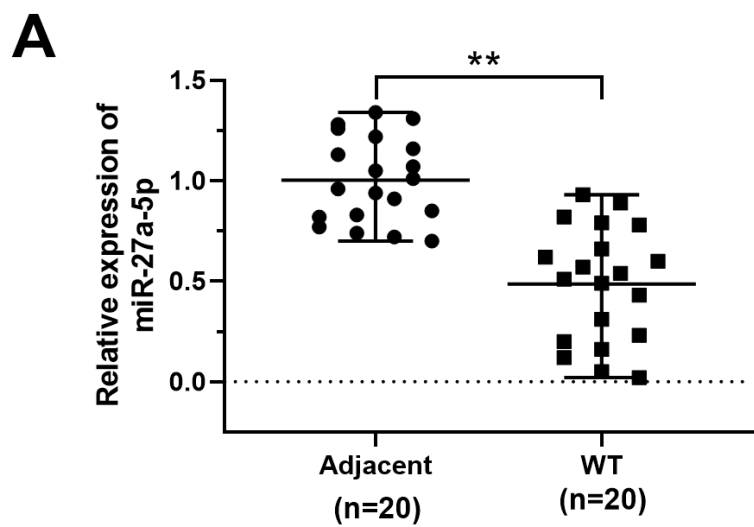
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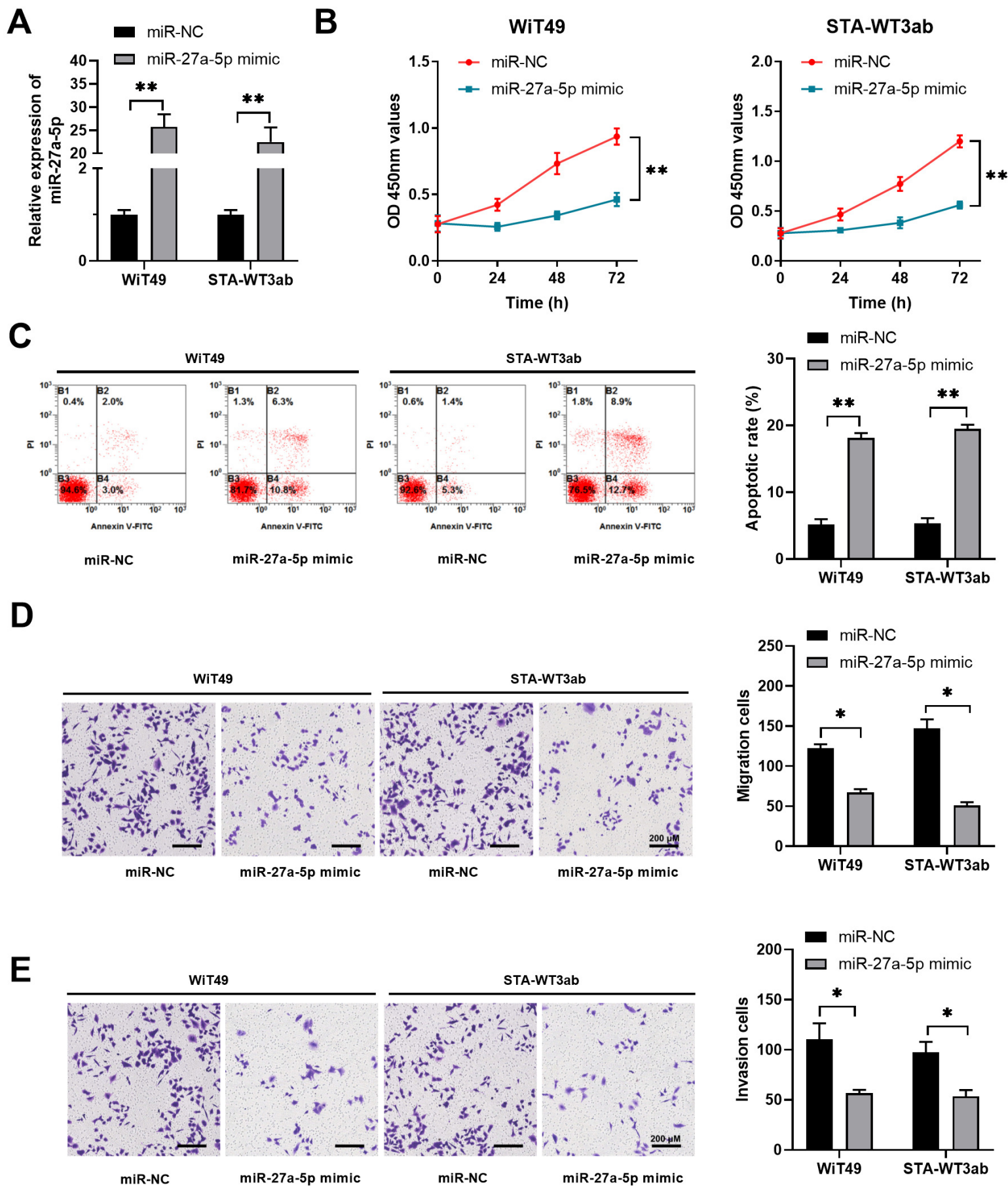
1 **Abstract**

2 Wilms tumor is the most common type of renal tumor in children. MicroRNAs
3 (miRNA) are small non-coding RNAs that play crucial regulatory roles in
4 tumorigenesis. We aimed to study the expression profile and function of miR-27a-5p
5 in Wilms tumor. MiR-27a-5p expression was downregulated in human Wilms tumor
6 tissues. Functionally, overexpression of miR-27a-5p promoted cell apoptosis of
7 Wilms tumor cells. Furthermore, upregulated miR-27a-5p delayed xenograft Wilms
8 tumor tumorigenesis in vivo. Bioinformatics analysis predicted miR-27-5p directly
9 targeted to the 3'-untranslated region (UTR) of PBOV1 and luciferase reporter assay
10 confirmed the interaction between miR-27a-5p and PBOV1. The function of PBOV1
11 in Wilms tumor was evaluated in vitro and knockdown of PBOV1 dampened cell
12 migration. In addition, overexpression of PBOV1 antagonized the tumor-suppressive
13 effect of miR-27a-5p in Wilms tumor cells. Collectively, our findings reveal the
14 regulatory axis of miR-27-5p/PBOV1 in Wilms tumor and miR-27a-5p might serve as
15 a novel therapeutic target in Wilms tumor.

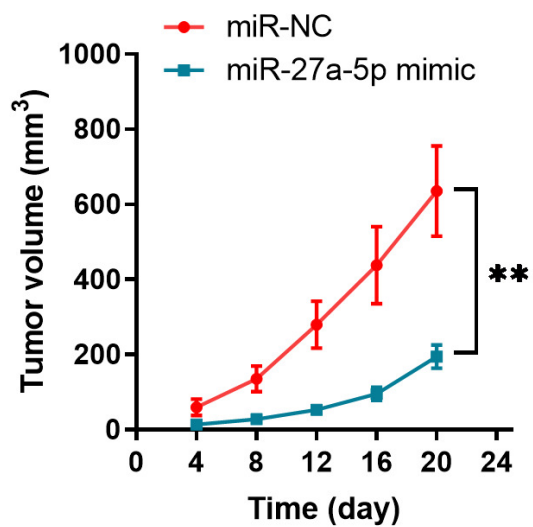
16 **Key words:** microRNA-27a-5p, Wilms tumor cell, PBOV1, biomarker

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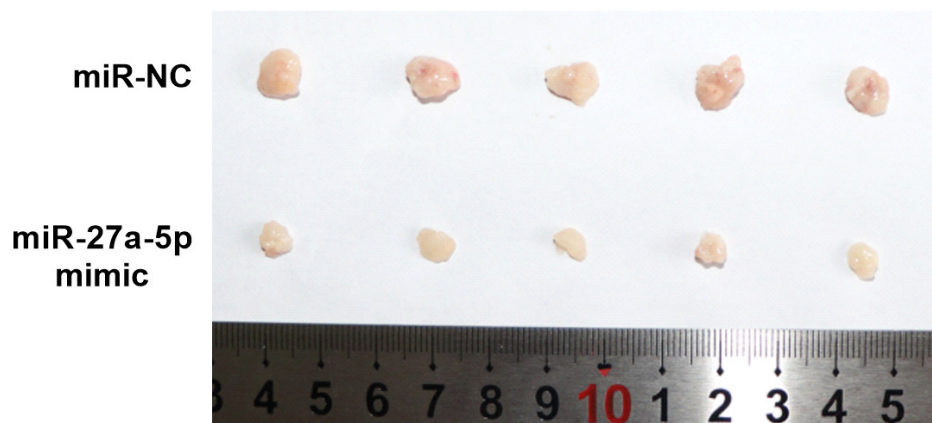




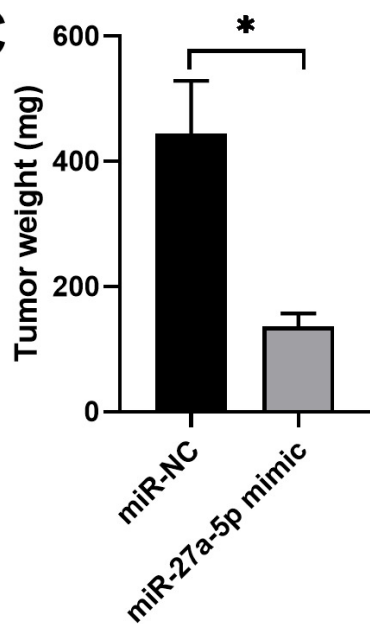
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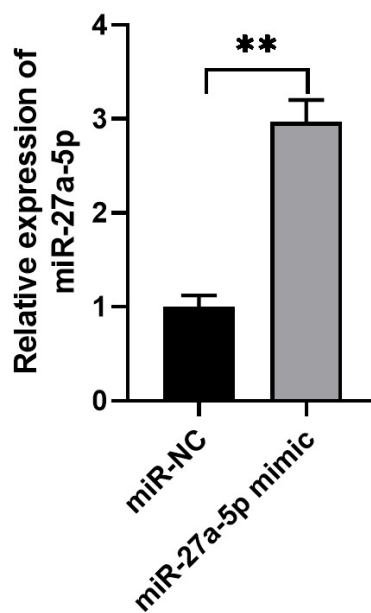
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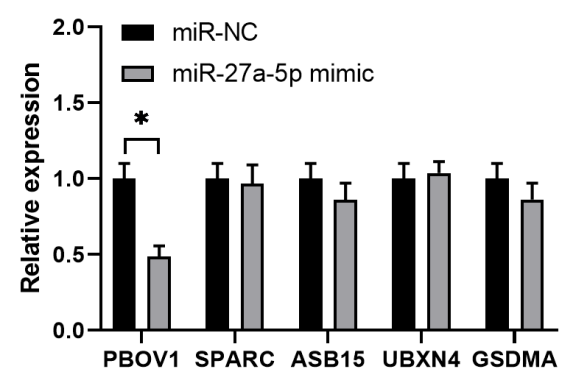
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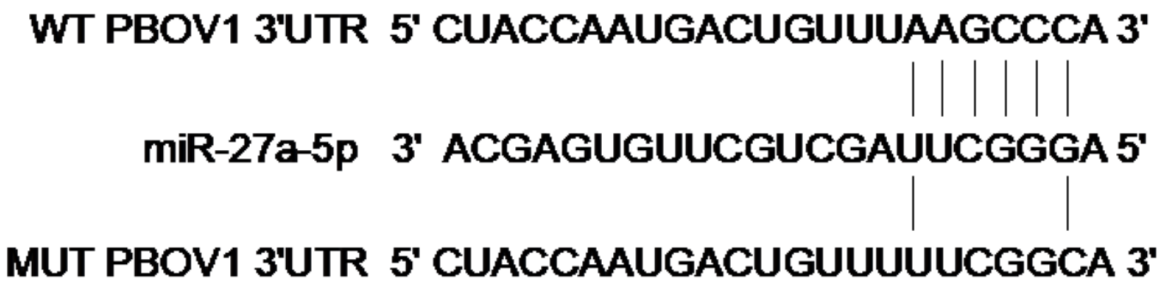
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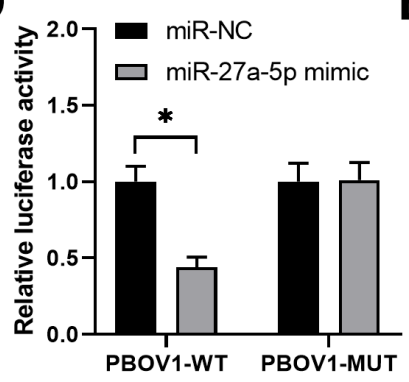
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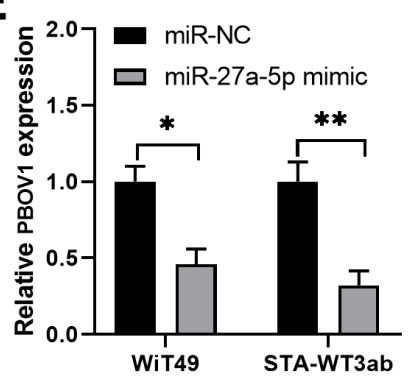
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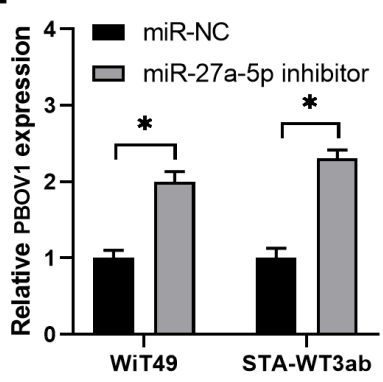
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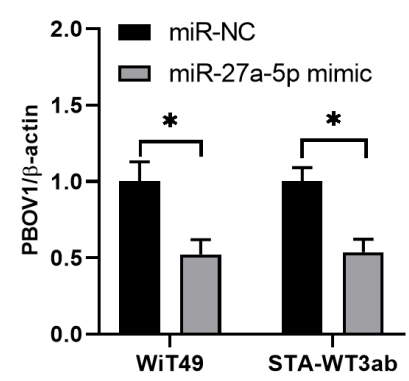
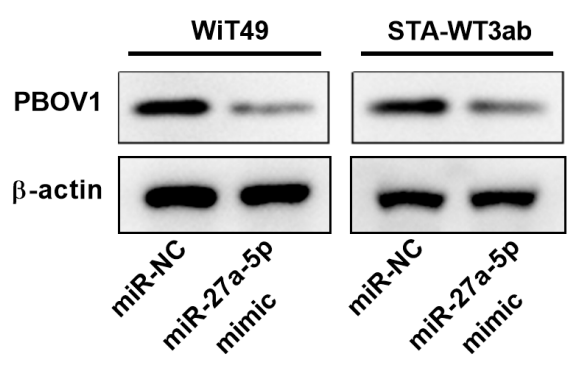
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